March 16, 1899.

The LORD LISTER, F.R.C.S., D.C.L., President, in the Chair.

A List of the Presents received was laid on the table, and thanks ordered for them.

The CROONIAN LECTURE, "On the Relation of Motion in Animals and Plants to the Electrical Phenomena which are associated with it," was delivered by Professor BURDON SANDERSON, F.R.S.

The following Papers were read:—

I. "Experiments in Micro-metallurgy: Effects of Strain." By Professor EWING, F.R.S., and W. ROSENHAIN.

II. "On Transmission of Proteosoma to Birds by the Mosquito: a Report to the Malaria Committee of the Royal Society." By Dr. C. W. DANIELS.

The Society adjourned over the Easter Recess to Thursday, April 20th.

"On Transmission of Proteosoma to Birds by the Mosquito: a Report to the Malaria Committee of the Royal Society."

By Dr. C. W. DANIELS. Communicated by Dr. M. FOSTER, Sec.R.S., by direction of the Malaria Committee. Received February 13,—Read March 16, 1899.

I have the honour to report the results of my observations since my arrival here (Calcutta) on December 21, 1898.

2. Major Ronald Ross, I.M.S., after demonstrating and explaining to me his method of dissecting the mosquito, showed me in prepared specimens the pigmented bodies met with in the stomach walls of mosquitoes fed on birds infected with Proteosoma, and also the changes which these bodies undergo day by day. Finally he demonstrated to me the "germinal threads" in cysts in the stomach wall, in the fluids of the body, and in the cells of the veneno-salivary glands.

3. On my arrival there were in the laboratory, in test-tubes, several series of mosquitoes which had fed on birds infected with Proteosoma on the nights of November 30, December 10, December 12, December 15, and December 20.

Of each of these series Major Ross dissected specimens, and demonstrated in them the same bodies that he had already shown me in prepared specimens. He pointed out that in the older mosquitoes it was possible to predict from an examination of the fluid obtained on cutting the thorax the nature of the contents both of the "coccidia" (the term employed by Ross)* in the stomach, and of the cells of the veneno-salivary glands.

These points I readily observed.

4. Of the mosquitoes referred to I day by day examined those which died, and others which I killed. In these I was able to repeat the observations and, in insects belonging to the earlier series, to trace the changes in the size and in the nature of the contents of the "coccidia."

I also examined a large number of mosquitoes caught about the laboratory, and others which had been raised from larvæ. In none of these did I find either "coccidia" in the stomach wall, germinal threads in the body fluids, or germinal threads in the cells in the salivary glands; nor did I find "black spores" (Ross).

5. Major Ross informed me that his published results were based on observations made in the hot season, when the temperature was 80° F., or over; and that now, as it was the cool season, I should find the changes progress more slowly, although the sequence of events was the same. My observations on the mosquitoes fed on December 20 and December 15 showed that this was the case. Major Ross also informed me that, with the lowered temperature, mosquitoes fed less readily, and that more difficulty was experienced in rearing them to a spore-bearing age.

These difficulties the use of the incubator was only partially successful in overcoming.

6. On the evening of January 1, following exactly in Major Ross's lines, I commenced a repetition of his main experiment:—

A large number of grey mosquitoes, reared from larvæ, were released in two mosquito nets.

In net No. 1 four birds were placed. On December 31 I had already found Proteosomata in large numbers in three of these birds, and in the fourth in moderate numbers.

In net No. 2 two birds, in whose blood no Proteosomata had been found, were placed. These two birds died two and three weeks later; on dissection no black pigment was found in their organs. Repeated examinations of their blood had failed to discover Proteosomata.

On January 2 none of the mosquitoes had fed, and on January 3 only two in net No. 1 and eight in net No. 2. On January 4, which was a warm night with a minimum temperature of 59·2° F., sixty-three mosquitoes were found in the morning gorged with blood

* See note by the Malaria Committee appended to this Report.

in net No. 1, and were caught in separate test-tubes which were then plugged with wool and placed in the incubator. Of the control series in net No. 2, where the non-infected birds had been placed, eighteen were caught and treated in the same way.

On the following two evenings, with minimum temperatures of 60·7° and 63·2°, sixty-three and forty-six mosquitoes were fed on the infected birds, net No. 1, and were kept for the preparation of specimens; and twelve mosquitoes were fed on the non-infected birds, net No. 2, bringing the number of the control series up to thirty-eight. At a later date eighteen mosquitoes were fed on a blue jay with numerous halteridia.

On the third day the sixty-three mosquitoes, from net No. 1 (with exception of those previously killed for examination or which had died) were released inside a clean net free from other mosquitoes. Birds free from Proteosoma were also placed in this net.*

In the morning all mosquitoes found inside were collected. Most of them had fed well. The minimum temperature during the night had been 63·2° F.

The mosquitoes were not fed on the following night as they were full of blood which most of them voided during the night. Many died next day.

The remainder were given the opportunity of refeeding every night after this; but as a spell of cold weather set in with minimum temperature of 44—49° F. (only on one night did it exceed 50° F.) few fed well or at all, and there was a consequent continued heavy mortality. Only one insect, which subsequently escaped in the night, being alive on the 10th day.

This method of feeding was very unsatisfactory in exceptionally cold weather. During the day the mosquitoes being kept warm in the incubator rapidly digested their food, whilst at night the cold rendered them torpid and they did not feed.

The control mosquitoes, of net No. 2, were treated in exactly the same manner, being fed on birds free from Proteosoma. The last died on the 13th day.

7. The results of the two series are as follows:—

Of sixty-three mosquitoes fed on proteosomal birds, forty-nine were

* This is the method Ross employs to re-feed mosquitoes. If infected birds are employed to re-feed the insects, a younger generation of coccidia is produced; I therefore used sterile birds for this purpose.

The method works fairly well in warm weather, but there is always some loss, as the full number is never collected again in the morning. As the process is repeated over and over again, this loss becomes serious, the more so the longer the period required for maturation of the coccidia. Moreover, in a frequently repeated process of this kind there is always the possibility of an outside mosquito getting inside the net, and to that extent vitiating the experiment.

examined, three were reserved for sections, one was too much decomposed for satisfactory examination; ten were not accounted for, having been lost in the nets.

Of the forty-nine examined two were killed on the first day,—that is, under twenty-four hours, and possibly under twelve hours after they had fed; no coccidia were found in these. Two more were examined the following morning, that is under thirty-six and possibly under twenty-four hours after they had fed; no coccidia were found in these.

In two examined about 4 p.m. of the same day, that is, under forty-six and possibly not more than thirty-four hours after they had fed on the infected birds, minute pigmented coccidia were found.

The remainder were examined on the following days. The largest numbers (eighteen) were examined on the fourth and (twelve) on the seventh days, as on these two days the mortality amounted to this.

In all these mosquitoes, with one exception, coccidia were found—usually in numbers; in one there was only one coccidium.

The exception occurred on the ninth day; but as by that time the insects had been re-fed several times, the mosquito in question may have been an outside one which had effected an entrance.

Of forty-five mosquitoes fed on the infected birds and examined, more than thirty-four hours after, forty-four contained coccidia.

This I may say is a more successful result than in the other series I have seen.

The other two series of mosquitoes were used by all of us for the preparation of specimens, and no record was kept of the number of non-infected insects. Judging from my own examination, only about three-quarters of them developed coccidia. Their treatment had been somewhat different, as for several days half of them were not incubated.

Of the controls fed on birds free from Proteosoma, thirty-eight in number, and treated in the same manner, twenty-nine were examined and nine are unaccounted for—lost in the nets. None of the twenty-nine were examined on the first day, but one was on the afternoon of the second day. The largest number, seven and five, were examined on what would correspond to the fourth and seventh days, four were examined on the fifth and four on the sixth days.* In none of these twenty-nine were coccidia found.

Of the eighteen fed on the blue jay with halteridia, twelve were examined from two to six days after feeding; none contained coccidia.

* It will be observed that these control mosquitoes were not, as the other series, collected on one, but on three nights. A very slight difference in breeze and light seems to affect the numbers that feed; any extra restlessness on the part of the birds has the same result.

8. The coccidia (pigmented bodies) found on the second day measured 6—7 μ, some of them a little more. They were oval bodies containing scattered granules of black pigment, and had a sharp, clear outline.

I incised the stomach of infected mosquitoes and by repeated washing and compression with a cover glass was able not only to wash out the contents, but even to express the loosely attached epithelium, so as to leave the stomach a transparent clear bag. The majority of coccidia remained fixed to the outer wall, though in one of the mosquitoes I observed a few coccidia escape with the epthelium. On subsequent attempts to detach the coccidia by this process I failed to do so, though some coccidia would be ruptured.

The next morning the smallest coccidia measured 10 μ; some were 12 μ. On the sixth day they were met with up to 30 μ; by this time the pigment had absolutely as well as relatively diminished.

In another three days some of them reached 60 μ; and in the last of the series examined (tenth day), there were coccidia measuring 70 μ.

The coccidia could now be seen to project from the outer wall of the stomach; very few contained pigment, and that only in small amount.

Some of the coccidia were clear, and others had a granular appearance; but in none were either black spores or germinal threads to be seen.

9. For the observation of the further development of the coccidium the early deaths of the mosquitoes, owing to the inclemency of the weather, rendered this series useless.

One of the insects infected on the night of January 5, and another infected on January 7, did reach this more advanced stage; and in the last of those fed on January 5, and which died on January 22, ruptured cysts, as well as numerous cysts containing mature germinal threads were found by me in the stomach wall, these threads were also found in the body fluids and in cells in the salivary glands. In one of the mosquitoes infected on January 5, which died on January 19, the coccidia had an appearance of striation.

In consequence of the effects of the unfavourable climatic conditions on the experimental insects, my observations on the development of the proteosomal coccidium were mainly made on mosquitoes infected November 30 and subsequent dates before my arrival, and on some infected on December 22.

On adding salt solution (15 grs. to the ounce) to an ordinary slide containing an infected mosquito stomach, and pressing on the cover glass, a projecting coccidium was ruptured; the contents poured out into the fluid, leaving the cyst wall still attached to the stomach.

The contents were seen to consist of a mass of shrivelled threads.

This appearance I frequently observed in the other series of infected insects already mentioned.

These threads, Ross's germinal threads, are sickle-shaped bodies, about 14 or 15 μ in length. They stain with logwood or methyl blue, but not strongly. On adding water or Farrant's solution they lose their shrivelled appearance, and become more rounded. Nearer one end than the other is an unstained portion (? nucleus). They show no signs of movement; but as they are invisible in water, and only become visible when shrivelled by the salt or stained, it may be doubted if they have been seen alive.

If the thorax of the mosquito at a somewhat more advanced stage in the development of the proteosomal coccidium is incised, similar threads will be found in the fluid exuded, if salt solution is added. In this case ruptured cysts can be found in the stomach wall.

The relation of the infection to the veneno-salivary gland involves a difficulty not met with in any other part of the examination.

The dissection of the stomach is easy; that of the salivary gland in its entirety is not, and for some reason appears to be more difficult in the old infected mosquitoes. Any rough manipulation results in the detachment of the cells, and little more than the duct is left. In most cases, however, even in old infected mosquitoes, one entire gland, or portions of both, can be exposed in fair condition.

In every case where this was done, and in which germinal threads were found in the body-fluids, the germinal threads were also found in some of the cells of the salivary gland. I failed to find similar threads in the large number of salivary glands obtained from uninfected mosquitoes bred from larvæ, or caught about the laboratory, or from mosquitoes at the earlier stages of proteosomal infection.

The affected cells, as they have a granular appearance, can be distinguished with a low power; the unaffected cells are quite clear.

With a high power, if not very numerous, the isolated germinal threads can be clearly distinguished in the cells; they are recognised by their peculiar shape and shrivelled appearance (the examination must be made in salt solution). If numerous, the individual threads can be better made out in the cells of the salivary gland than in the coccidia of the stomach wall; but, as in the case of the latter, pressure on the cover glass will rupture the cell, and the germinal threads are then poured out.

The threads do not fill the cell. There is a faintly granular crescentic portion on the side most remote from the duct which, in many cases at least, is free from threads. The part of the cell in which the threads lie must be nearly fluid, as it permits oscillation of the threads to take place.

The whole of the veneno-salivary gland is never involved. In one dissection made by Ross the cells in both middle lobes and in no other

part of the gland contained the threads. In several instances, where one gland has been exposed entire, the middle lobe alone has been involved; but in the majority all that can be stated with certainty is that the cells in one portion of the gland contain threads, and that those in other portions do not.

On these points I have satisfied myself by repeated examination, though the appearances are by no means difficult to make out.

I have gone at some length into the description of this matter, as, so far, we have found no satisfactory method of making permanent preparations. All the preservatives at our disposal, with the exception to some extent of weak formalin solution, wrinkle up the delicate cells; and I have no confidence in this agent as a means of making permanent specimens.

The following specific observations made by myself on mosquitoes dissected by Major Ross, Dr. Rivenberg, of the American Mission, who is working with Dr. Ross, and myself may be of interest:—

(*a*) Coccidial cysts full of apparently mature germinal threads; no ruptured cysts; no germinal threads in the body-fluids or salivary glands. Two observations.

(*b*) Cysts full of germinal threads; other ruptured empty cysts; germinal threads in body-fluids; germinal threads in salivary glands. Over twenty observations.

(*c*) Empty cysts in stomach wall; germinal threads in body-fluids of thorax; germinal threads in salivary glands; no cysts still containing germinal threads. Two observations.

(*d*) Empty cysts only in stomach wall; no germinal threads in body cavity; no germinal threads in well exposed salivary glands. One observation; the mosquito had been infected four weeks before death.

These observations fully confirm Ross's statement in every point. They indicate that the threads are formed in the coccidia; and that the germinal threads escape into the body cavity on the rupture of the coccidia, to be again collected in the salivary glands.

I should have liked to extend the series, but the continued cold weather renders it improbable that I shall be able to do so before I leave.

10. The infection of birds free from Proteosoma by the bites of mosquitoes.

On December 20, the day before my arrival, twenty-two birds were examined and found free from Proteosoma. On that night some of these birds were used for feeding the mosquitoes which had been infected on November 30 (?) and on the 24th and subsequent days; the remainder of the birds were used for feeding the mosquitoes first infected on November 30 and December 10, 12, and 15. In other

mosquitoes of this series germinal threads were found in the salivary glands; and those which fed, when examined later, gave the results indicated in paragraph 9.

On December 30 Dr. Rivenberg and myself examined these birds; three of them had Proteosoma, two in large numbers.

On January 4 I examined them all except one which died on January 2; in this bird the heart's blood contained no Proteosomata, and the organs were free from pigment.

Five more of them had now Proteosoma; in every instance the parasites were very numerous. On January 6 and 7 I again examined them; three more had Proteosoma, also in large numbers.

On January 9 no more cases had developed; but on January 18 one of the birds had numerous Proteosomata. It was also ascertained that many of these birds which previously had been found to be infected had now recovered, whilst others showed but a few Proteosomata.

Thus twelve out of twenty-two birds (54 per cent.) became infected. This compares unfavourably with Ross's earlier results, as, in his published series, twenty-two out of twenty-eight (79 per cent.) were infected. But it is to be remembered that at the time this result was obtained the germinal threads were found at the end of a week; whilst in December the development was much slower, and took at least twice the time. It is much easier to keep mosquitoes alive during the first week after feeding them than it is to keep them alive for any subsequent period; moreover, in hot weather, such as Ross had worked in, mosquitoes bite more readily.

These results appear less unfavourable, if they are considered in connection with observations on the normal proportion of wild, uncaged birds, infected with Proteosoma at this season. Thus, earlier in the year, Ross, out of 111 wild birds, found Proteosoma in fifteen, or 13·5 per cent.; whilst I found at this season only one out of thirty, or 3·3 per cent. affected with Proteosoma.

It is possible that in the cold season the birds have a greater power of resistance; the validity of this conjecture is rendered more probable by the short duration of the proteosomal attack in my infected birds. Of the twelve, five died within the first week. In three of the survivors, in which the Proteosomata had been very numerous, no parasites could be found ten days after the commencement of the invasion; in one in which they were never numerous none could be found on the fifth day. In the other three very few are now found, though at first they were numerous.

The recovery of these birds and the death of the mosquitoes fed on them diminishes the chances of much future work on this line during the time remaining to me here.

11. Mention has been made of the differentiation of the coccidia (previous to the formation of the germinal threads), according to the

appearance of their contents, into clear and granular; the evolution of the latter into the coccidia containing germinal threads can be traced day by day. This differentiation was clearly visible in my series.

In a minority of the coccidia, and in most infected mosquitoes, when the germinal threads are mature, certain black tubular bodies are to be found in cysts with otherwise clear contents. These black tubular bodies were frequently met with in the series of mosquitoes infected in November and December. Most of these mosquitoes contained some coccidia with black tubular spore-like bodies; though in a few insects all the cysts contained germinal threads only. In some cysts the black spores were numerous, and occupied the entire cyst; in other cysts there were only a few. In most instances germinal threads were not found in the black spore-bearing cysts; but there were a few such cysts in which it was doubtful whether germinal threads were present or not, or whether the appearance arose from over-lying threads which had escaped from a neighbouring capsule.

These black spores are very resistant; I have seen some which had been kept in water for months by Ross, and which had undergone no visible change. They withstand irrigation with liquor potassæ.

When the cysts are ruptured the black spores are to be found all over the body of the mosquito, but not included in cells. They do not seem to accumulate in any particular organ.

The most plausible view of the nature of these black spores seems to be that held by Major Ross, viz., that they are "resting spores," and that through them, by another cycle, the Proteosoma can be propagated in conditions unfavourable for direct propagation by mosquito-insertion into a warm-blooded animal.

If this be the case, three courses suggest themselves:—

(*a*) From the black spores may arise bodies capable of non-parasitic life (and possibly of reproduction), which at certain stages of their existence, and in certain conditions, on introduction into a warm-blooded host by inhalation, through drinking water, or even by injection by a mosquito or other blood-sucker in transferring them from the medium in which they live, may resume parasitic habits.

(*b*) That they may be ingested by mosquito larvæ, and in them undergo such development as will result in the formation of germinal threads in the adult mosquito, which, in turn, may be injected into the appropriate bird.

(*c*) That they may, if swallowed or inhaled by an appropriate warm-blooded host, so develop as to reach the circulation and pass into the sporulating phase.

Such experiments as have been made on this subject are inconclusive; and it is obvious that until the nature of these "black spores" is

determined we cannot exclude, even for Proteosoma of sparrows, the possibility of any one of the many possible alternative channels of infection. Intervention of the mosquito intermediate host may be only an occasional requirement.

Still less are we justified in concluding that malaria in man can only be acquired through and directly from the mosquito; or in devoting our attention exclusively to that channel.

12. I have made myself familiar with the Proteosoma in sparrows, and the Halteridium in pigeons and crows.

In one specimen of a "blue jay," also, I found a very abundant Halteridium infection; the parasites in this instance had some peculiarities which I hope to work out if we can procure more of these birds. The bird I had died before I had completed my observation; I have preserved the organs as well as specimens of the blood in the heart.

13. In the cardiac blood of this jay there were numerous filariæ. They were sheathless, sharp tailed and fairly active, and had locomotory movement. They were of two sizes; in the shorter the tapering of the tail was much more abrupt than in the longer. Neither showed any extension or contraction.

Adults of one species only, three females and five males, were found in the subcuticular connective tissue, and in that round the trachea.

They were much longer and thicker than *Filariæ clava* (Wedl) or than the filaria described by Mazzini in the pigeon.

The females have the usual double ovary terminating in a vagina which appears tubular near the vulva situated near the caudal end of the body. The mouth is terminal and unarmed; the anus is subterminal.

The male has two spicules of equal length. The thickness of these worms, and the fact that when placed in weak formalin (2 per cent.) the cuticle burst in its entire length, will make them suitable for determining some of the disputed points in the anatomy of the Filaridæ.*

14. The difficulties in connection with human malaria are increased by the present plague scare. The suspicion of the natives about inoculation, makes them averse to any intercourse with European medical men.

By rewards however we have been able to get two fair cases of tertian fever, and three cases with crescent plasmodia—two of them with crescents in considerable numbers. On these cases we have fed mosquitoes—the common grey, and two varieties of "dapple wings"

* Judging from the description of the embryos, it is probable that these blood-worms of the Indian blue jay are identical with those found by Manson in Amoy, China, in the magpie (*Pica media*) and the gray mina (*Gracupica nigricollis*), in which case the mature form of one will be found to lie in the pockets of the aortic and pulmonary semi-lunar valves (*vide* 'Journ. of the Queckett Micro. Club,' vol. 6, p. 130, No. 44, August, 1880).

(large and small) in most points closely resembling those in which Ross had previously found pigmented cells after feeding on a patient with crescents. So far our results have been negative; but, in view of the peculiar climatic conditions, and of the possibility of the first stage, that of formation of coccidia, being inhibited by the cold, we are not prepared to accept these results as conclusive.

15. With Major Ross, I have examined the organs of some persons (eight) who died of kala azar. This appears to be an infectious disease, indistinguishable at first from malaria. Chronic in character, it continues for months and becomes associated with enlargement of the spleen and liver, and progressive anæmia. The present opinion of most of those who have been deputed to investigate kala azar, as well as of those with longest and most intimate experience of the disease, is strongly in favour of the view that it is malarial in origin.

The melanin or black pigment was absent in the organs of some of the cases I examined; but in all but one yellow pigment was present in the liver, and in most in the kidneys and spleen also, indicating hæmolysis. The iron reaction with acidified potassium ferrocyanide was obtained in the spleen in three instances and, in one, in the liver also.

So abundant and chronic a hæmolysis in cases of malaria, continued moreover after the parasite has ceased to be present (at any rate in sufficient numbers to be found in the peripheral blood or to cause appreciable deposit of melanin in the organs), raises the important question as to the possibility of the differentiation of parasites, with imperceptible morphological differences, by their toxic or hæmolytic properties.

16. Hæmoglobinuric fever seems to have been fairly common of late in some parts of India. I am collecting information, and have requested the editor of the 'Indian Medical Gazette,' to insert in that Journal a series of questions on the subject. Hæmoglobinuria does not occur in kala azar notwithstanding the great amount of hæmolysis which takes place in that disease.

I regret the length of this report, but the main subject of it, Major Ross' researches, cannot be dealt with in a few words, as they supply a basis for our future operations.

[It is necessary to point out that the word "coccidium" has been used by Major Ross and in Dr. Daniel's report above printed in a peculiar and not readily intelligible sense. "Coccidium" is the name of a genus of Sporozoa established by Leuckart in 1879 for the cell-parasite of the rabbit's liver, called *Coccidium oviforme*, and other allied species. "Proteosoma" is the name given by Labbé to another genus of Sporozoa parasitic in the blood-cells of birds. When Major Ross states in his report, dated May 21, 1898, that certain "parasites are

a development in the mosquito of Proteosoma in birds; and to judge from their structure and mode of growth so far as yet observed, I take them to be *coccidia*," he is using the generic term "coccidium," to describe some phase in the growth of the species of a distinct genus, Proteosoma.

Apparently, what Major Ross intends to indicate by the term "coccidium" is an ovoid firmly walled corpuscle which increases in volume from about 1/2000th inch in length to four or five times that size, and then breaks up into a mass of filiform spores radiating from a central granular mass.

In this mode of spore formation these bodies have resemblances to the true coccidia, which present themselves not only as oviform corpuscles but as cysts with sickle-shaped or filamentous spores. It is, however, not legitimate to apply the generic term "coccidium" to a phase of growth of another genus.—LISTER, *Chairman of the Malaria Committee.*]